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厦门大学

# 博 士 学 位 论 文

# GCN5 通过调控SRC-3促进肝细胞癌发生

# GCN5 potentiates Hepatocellular Carcinoma by Promoting SRC3 gene expression

**Sidra Majaz**

指导教师姓名：俞春东　教授

专　业　名　称：细胞生物学

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## ABSTRACT

Histone acetylation plays a central role in establishing an active chromatin environment for transcriptional regulation. The balance between histone acetylation and deacetylation is maintained in cells by histone lysine acetyltransferases (KATs) and histone deacetylases (HDACs). Imbalance between KATs and HDACs leads to altered acetylation states of genes which are associated with tainted state of cells resulting in cancer. HDAC inhibitors have been successfully tested as useful tool for cancer prevention and therapy. However such inhibitors often results in unintentional gene activation and the development of drug resistance. To ensure complete balance and control of histone acetylation in a specific manner the function of KATs in cancer requires thorough scrutiny.

GCN5, was the first KAT to be related with transcription, and is a crucial component of a transcriptional regulatory complex. GCN5 has been implicated in vast range of cellular functions from cell proliferation and differentiation to DNA damage and regulation of cell cycle. However with such conspicuous attributes in cell functions its role in cancer has been meagerly investigated and its involvement in cancer growth is still vague.

Our study revealed that expression of GCN5 is up-regulated in human hepatocellular carcinoma (HCC) tissues and cell lines. Studies in GCN5 knockdown stable cell lines elucidated that GCN5 knockdown can inhibitcell proliferation, G1/S phase transition in cell cycle and colony formation. GCN5 knockdown attenuated cell migration as demonstrated by wound healing assay. Besides that GCN5 regulated several cell cycle related and onco-proteins such as SRC-3, cyclinD1 and β-catenin. Our data showed that GCN5 can transcriptionally regulate SRC-3 expression in HCC cell lines through Akt pathway. GCN5 can also regulate SRC-3 2k promoter in a dose dependent manner and can combine with SRC-3 and E2F1 to enhance SRC-3 promoter activity. The tumor xenograft experiments in nude mice divulged that GCN5 knockdown cells produced significantly smaller tumors as compared to HepG2 control cells. These results indicate an oncogenic role of GCN5 in HCC and require further investigation into the mechanisms and pathways leading to tumor development. Moreover GCN5 inhibitors can be tested as potential tool for cancer therapy and prognosis.

Key words: GCN5, SRC-3 Hepatocellular carcinoma.

## 摘要

组蛋白乙酰化在转录调节过程中起着重要的作用，它可以使染色体处于一种活化的状态。在细胞中，维持组蛋白的乙酰化和去乙酰化之间的平衡是通过组蛋白乙酰转移酶（HATs）和组蛋白去乙酰化酶（HDACs）这二者间的平衡实现的。如果这二者间的平衡被打破了，那么基因的乙酰化水平就改变了，从而可能诱发癌症。虽然HDACs抑制剂可以有效的预防和治疗癌症，但是一些抑制剂会激活一些非靶基因从而使人出现抗药性。为了确保乙酰化和去乙酰化之间的完全平衡以及掌握在癌症中HATs在组蛋白乙酰化过程中特定的功能，HATs的功能仍需要进一步地研究。

GCN5，是发现的第一个和转录相关的组蛋白乙酰转移酶，而且它是转录调节复合物中的一个重要的组分。在相当多的细胞功能调节过程中都可以看到GCN5的身影，比如说从细胞增殖、分化到DNA损伤再到细胞周期的调节。但是在癌症的发生发展中，却很少有报道GCN5在其中发挥了什么作用。

我们的研究表明，在人肝细胞癌（HCC）的组织和细胞系中，GCN5都是过表达的。在GCN5稳定敲低的细胞系中发现，敲低了GCN5可以抑制细胞的增殖和细胞克隆形成的能力，在细胞周期中可以抑制G1/S期的转变。并且通过划痕实验分析我们发现，敲低GCN5可以降低细胞的迁移率。除此之外，GCN5可以调节一些细胞周期相关的原癌蛋白，例如SRC3。我们的数据显示，在HCC细胞系中，GCN5可以通过Akt信号通路在转录水平上调节SRC-3的表达。GCN5还可以以浓度依赖的方式来调节SRC-3 2k的启动子活性，通过与SRC-3和E2F1结合来进一步增强SRC-3启动子的活性。从裸鼠成瘤实验我们可以得出，敲低GCN5的细胞形成的肿瘤要明显小于对照细胞形成的肿瘤。以上的这些结果都可以反应出，GCN5在HCC中是以一个原癌基因的身份存在着，它在肿瘤发展过程中的的机理和相关通路有待于进一步的研究和探索。此外GCN5的抑制剂可以作为一种潜在的治疗癌症的手段。


**关键字**：GCN5，SRC-3，肝细胞

# CHAPTER 1

# INTRODUCTION

## 1.1. CHROMOSOME:

Schleiden, Virchow and Bütschli were among the first scientists who recognized the structures now described as chromosomes. The term was coined by von Waldeyer-Hartz, referring to the term chromatin, which was introduced by Walther Flemming [1]. Although chromosome is present in both prokaryotes and eukaryotes, however prokaryotic chromosome differs from eukaryotes in being less sequence based structured and circular with a single strand and organized in a structure called nucleoid whereas in eukaryotic cells the chromosome exists within nuclei such as those found in plants, yeast, and animals[2]. Since our focus is on the eukaryotes so details of eukaryotic chromosome will be discussed only. Eukaryotes possess multiple large linear chromosomes contained in the cell's nucleus. Each chromosome has one centromere, with one or two arms projecting from the centromere, although, under most circumstances, these arms are not clearly visible. In addition, most eukaryotes have a small circular mitochondrial genome, and some eukaryotes may have additional small circular or linear cytoplasmic chromosomes. In the nuclear chromosomes of eukaryotes, the uncondensed DNA exists in a semi-ordered structure, where it is wrapped around histones (structural proteins), forming a composite material called chromatin.

### 1.1.1. Structure of Chromosome:

In all eukaryotic cells resides a nucleus which contains super coiled genomic DNA, constrained, called chromosome. The formation of chromosome takes place with the help of structural proteins called histone and compacted by histone and non histone proteins in a dynamic polymer which is accompanied with different transcription factors at the time of transcription and several other macro molecules. This composition of DNA with macromolecules and other proteins associated with it is called chromatin. The structure of chromatin is dynamic and varies significantly during different stages of cell cycle. Chromatin on its further packaging along with other associated molecules is called nucleosome. In each nucleosome, roughly two super helical turns of DNA wrap around an octamer of core histone proteins formed by four histone partners: an H3-H4 tetramer and two H2A-H2B dimers [3].

### 1.1.1 The Histone Code:

Nucleosome is the basic repeating unit of chromatin and responsible for its organization. Histones are small basic proteins consisting of a globular domain and a more flexible and charged NH2-terminus (histone "tail") that protrudes from the nucleosome. Such intricate structure suggests that chromatin structure plays a vital regulatory role and a converging point for multiple signaling pathways.

### 1.1.2 The Human Chromosome:

The human genome comprises of 46 chromosomes which is comprised of 22 pairs called autosomes and a pair of sex chromosomes having XX chromosome in females and XY chromosome in males. Certain genetic traits are linked to a person's sex and are passed on through the sex chromosomes. The autosomes contain the rest of the genetic hereditary information. All act in the same way during cell division [4]. Hence it would not be wrong to say that human chromosomes play a major role in transfer of genetic information from parents to offspring and thus is the basis of hereditary. Moreover aberration in human chromosomes has been linked with several syndromes and diseases including cancer. Thus post translational modifications and mutation including physical damage to chromosomes can be leading cause in disease prevalence in Humans.

## 1.2 EPIGENETICS:

Genomic DNA is the physiological template of all eukaryotic genetic information and ultimate template of heredity. However DNA transcription is frequently modified by diverse array of posttranslational modifications that particularly impinge on histone amino termini, thereby regulating access to the underlying DNA. Specific histone modifications can generate synergistic or antagonistic interaction affinities for chromatin-associated proteins governing the transition between transcriptionally active or silent chromatin states. This epigenetic marking system represents a fundamental regulatory mechanism that impact majority, if not all, transcriptional processes, with diverse outcomes for cell fate decisions during normal and pathological development [5, 6].

### 1.2.1 Epigenetic Modifications:

Epigenetic modifications can play a vital role in cells from transcriptional regulation to metabolic functions. Major regulation of protein function is achieved by covalent

posttranslational modifications of proteins [7, 8]. Covalent modifications of histones can regulate all DNA-dependent processes. In the past decades, it has become conspicuous that histone modifications are key players in regulation the states and dynamic of chromatin as well as in gene expression. Therefore, the enzymatic machineries that set many sites and types of histone modifications are vital regulators and provide a wealth of variable combinations which in turn provides huge regulatory potential for remodeling chromatin states, that facilitate or inhibit gene transcription, DNA replication, repair or recombination, which in turn can control cell proliferation, differentiation, plasticity, and malignancy processes [9, 10].

### 1.2.2 Types Of Modifications:

Histones contain a globular domain that promotes histone–histone interactions within the nucleosome and also provides a binding surface for DNA. In addition, they contain tail domains that protrude out of the nucleosome, where they influence histone–histone interactions, interactions between histones and DNA, and between histones and other proteins. Although both the globular domains and the tail domains can be modified, the histone tails are particularly rich in modifications, including methylation, acetylation, phosphorylation, ubiquitination and sumoylation [10]. Here lysine acetylation has been described in detail.

### 1.2.3 Lysine Acetylation:

Lysine acetylation is a reaction in which an acetyl group from the acetyl coenzyme A (acetylCoA) cofactor is transferred to the e-amino nitrogen of lysine residues. This reaction is catalyzed by histone acetyltransferases (HATs), whereas the reverse reaction is performed by histone deacetylases (HDACs).Vincent Allfrey proposed more than 3 decades ago, that histone acetylation was associated with transcriptional activation in eukaryotic cells [7, 11] Acetylation at specific lysine residues on particular histones can aid the binding of regulatory factors involved in the transcription process at specific steps. Both individual nucleosomes and higher-order chromatin folding can block access of RNA polymerase and other factors to gene promoters. Acetylation affects chromatin folding as the addition of the acetyl group neutralizes the positive charge of the lysine, weakening bonds between histones and the negatively charged DNA backbone, as well as the bonds between neighboring nucleosomes, allowing for more relaxed chromatin structures leading to gene transcription [12]. Besides that acetylated lysines can create a specific signal for regulatory factors or chromatin-remodeling

complexes and contribute to their targeting to a specific region. These finding suggest that histone acetylation can result in increased transcriptional activity in vivo. It has been found that bromo domains of histone acetyl transferase act as acetyl-lysine recognition modules, directing enzymes that contain them to particular chromosomal sites.[13, 14] Besides transcription, new roles for histone acetylation have been uncovered which includes nucleosome assembly, chromatin folding, heterochromatic silencing [15] DNA damage repair, and replication.[16-18].

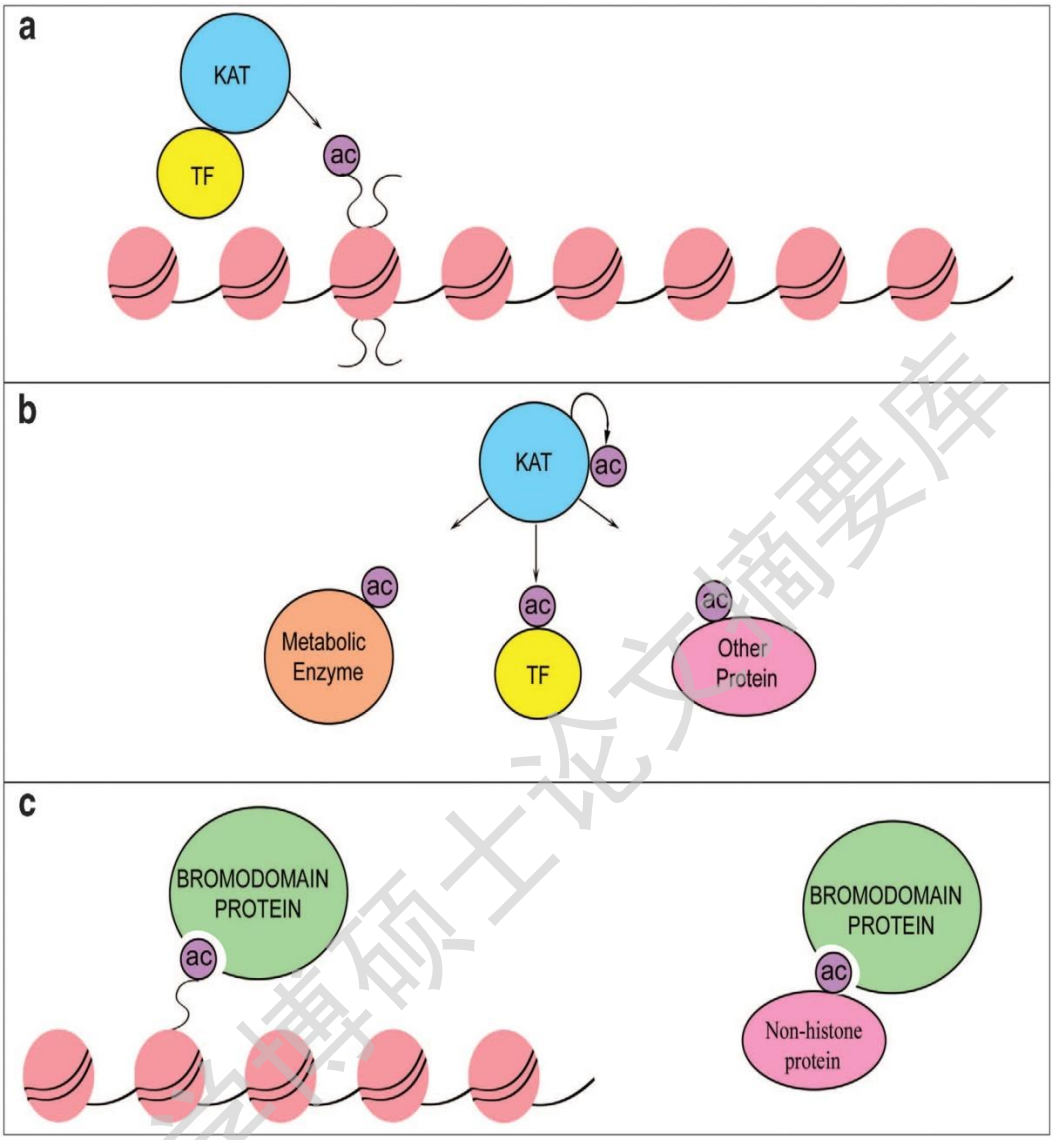


Figure 1. Mechanisms of action of acetylation. (a) KATs target both tails and globular domains of all four histone proteins. (b) KATs acetylate non-histone proteins including transcription factors (TFs) as well as metabolic enzymes and other nuclear and cytoplasmic proteins. (c) Bromodomain-containing proteins bind to acetyl-lysines on histone tails and on non-histone proteins.

From A Farria, W Li, and SYR Dent: KATs in cancer: functions and therapie [10]

### 1.2.4 HATs and KATs

Although originally termed histone acetyltransferases (HATs), due to their actions toward abundant histone substrates, lysine acetyltransferases (KATs) are located both in the nucleus and in the cytoplasm, and they have many non-histone substrates as well. The first HAT catalytic proteins were isolated and cloned in the 1990s [10, 19, 20]. The revolutionary finding

that a transcriptional coactivator protein, GCN5, is a nuclear HAT [21] led to a surge in HAT-related research and the identification of numerous enzymes and their substrates. HATs can be grouped on the basis of their catalytic domains into 3 families: GCN5 N-acetyltransferases or GNATs (including GCN5, PCAF, Hat1, and others); MYST HATs (named for the founding members of this family: MOZ/Morf, Ybf2, Sas2, and Tip60); p300/CBP; SRC (steroid receptor co-activator) including (SRC-1, SRC-2, and SRC-3) and an "orphan class" of HAT enzymes lacking a true consensus HAT domain, featuring, among others, p300/CBP and Taf1 [16].

### 1.2.5 Implication Of Kats In Cancer:

The fact that histone acetyl transferase can regulate gene transcription and associate with transcription factors, has led to momentous advances in dissecting the role of HATs in cancer. In past few years all the major HATs have been intimately linked with cancer progression. In addition to the HAT domain, Lysine acetyl transferase (KATs) possesses several domains that facilitate interactions with other proteins, including reader domains for acetylation and other modifications. Together these domains allow for specificity and diversity in KAT substrates. Substrates of these enzymes as well as their roles in biological functions are continuing to be defined. CBP/p300 have already been implicated in cancer development and progression [22-25]. To date all KATs probed have vital functions in cellular differentiation and embryo growth [26]. As many cancers fail to differentiate, it is likely to link KATs with tumorigenesis. Several KATs with their established and putative role in variety of cancer have been shown briefly in Table 1.

**Table 1:** Representative members of the families, with important physiological roles as well as putative links to carcinogenesis are shown.

| **Families** | Proteins | Associated complex | Substrate specificty | Structural features | Role in cancer | Ref |
|---|---|---|---|---|---|---|
| **GNAT** | GCN5 | STAGA, TFTC | H3, (H4, H2B) | Bromo domain | Contributes in NSCLC, ALL | [10, 16, 27] |
| | PCAF | PCAF | H3, H4 | HAT domain | Enhance prostrate and colon cancer. | |
| | Hat1 | HAT-B, NuB4, HAT-A3 | H4, H2A | WD40 | Putative role in HCC | |
| | ATF2 | | H2B, H4 | Motif A of HAT domain | Melanoma increased mRNA in gastrointestinal tumors, Skin tumors in mice. | |
| **MYST** | MORF & MOZ | MSL | H3 | MYST domain, Chromo domain | Chromosomal translocations of MOZ create bona fide oncogenes. | [10, 16, 28, 29] |
| | MOF | | | | Lung cancer. | |
| | Tip60 | NuA4 | H2A, H4 | | Tumor suppressor, putative role in oncogenesis. | |
| | HBO1 | ORC, MCM | H3, H4 | Zinc finger, MYST domain | Breast cancer, inhibitor of glioma and angiogenesis. | |